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Pro-dynorphin peptides are found in the same neurons throughout rat brain: Immunocytochemical study

(dynorphin/α-neo-endorphin/neuropeptide/neuroanatomy)

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ABSTRACT **It is known that the opioid peptide dynorphin A has a broad distribution throughout the neuraxis. Recent biochemical studies have extended the sequence of dynorphin A by 15 amino acids to include another [Leu]enkephalin-containing peptide known as dynorphin B. These sequence data have been validated by the elucidation of the structure of the hypothalamic mRNA coding for α- and β-neo-endorphin, dynorphin A, and dynorphin B. Using specific antisera directed against each of the three opioid peptides, we have studied their cellular distribution in rat brain. Their distribution patterns are extremely similar, if not identical. Furthermore, all three peptide immunoreactivities can be localized to the same cells in five nuclear groups throughout the brainstem—the supraoptic nucleus, the paraventricular nucleus, a group of cells in the lateral hypothalamic area, the nucleus parabrachialis, and the nucleus tractus solitarius. The sequence of a common precursor for dynorphin A, B, and α- and β-neo-endorphin was deduced from hypothalamic mRNA. The ability to localize all three peptides together within cells in widely placed nuclei strongly supports the use of the same biosynthetic precursor for the neo-endorphin and dynorphin peptides in other parts of the central nervous system as well.**

The [Leu]enkephalin-containing opioid peptide dynorphin was extracted from pituitary and its sequence was determined (1, 2); later, the same was done for dynorphin from gut (3). Immunocytochemical studies in brain and pituitary (4) localized dynorphin-like immunoreactivity to the vasopressin-producing cells (5) of the magnocellular, neurosecretory neurons of the hypothalamus (supraoptic and paraventricular nuclei) and the posterior lobe of the pituitary. An identical set of anatomical observations (6–8) was made for a second [Leu]enkephalin-containing opioid peptide, α-neo-endorphin. Further biochemical studies on dynorphin and α-neo-endorphin (9–11) revealed an extensive distribution throughout the central nervous system.

Recently, the sequence beyond position 17 of dynorphin was extended 15 residues (12). That region contains Lys-Arg (as residues 18 and 19) followed by a 13-residue [Leu]enkephalin-containing peptide with the following structure: (H)Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr(OH) [independently confirmed by Kilpatrick *et al.* (13)]. This peptide was named dynorphin B, and the original 17-residue dynorphin was renamed dynorphin A (12). The relationship between these three [Leu]enkephalin-containing opioid peptides has recently been explained by the mRNA sequence elucidated by Kakidani *et al.* (14) by using cloning methods for cDNA. Using poly(A)-rich mRNA extracted from pig hypothalamus, these workers showed that the pro-dynorphin precursor produces α-neo-endorphin, dynorphin A, and dynorphin B as well as various previously undescribed peptide sequences not containing enkephalin structures.

Although the evidence is conclusive regarding a common precursor for the three [Leu]enkephalin-containing peptides in hypothalamus, there is little biosynthetic information about these peptides in other brain regions. It is reasonable to expect a similar biosynthetic route for dynorphin A in the various known positive cell groups (15). However, this is by no means self-evident. For example, α-melanotropin-like immunoreactivity can be seen in two cell groups, one being β-endorphin-positive and the other not (16, 17). Such discrepancies have been attributed either to differential processing of the precursor or to different precursors.

In this paper we present immunocytochemical evidence that α-neo-endorphin, dynorphin A, and dynorphin B immunoreactivities occur within the same cells of several hypothalamic and caudal brainstem nuclei. These findings lead to the conclusion that pro-dynorphin (or a similar precursor) is found in many different cell types across brain and most likely is the source of the three peptides in various neuronal systems.

MATERIALS AND METHODS

Male Sprague–Dawley rats were injected by the intracerebroventricular route with 400 μg of colchicine 48 hr prior to sacrifice, perfused with 4% formaldehyde, and prepared for immunocytochemistry as described (4). Serial 5-μm sections were cut on a cryostat at −20°C through several levels of the hypothalamus, locus ceruleus, and nucleus tractus solitarius. The tissue was processed by the peroxidase-antiperoxidase method for immunocytochemical study as described (4).

Antisera against dynorphin A (R-1), dynorphin B (13S), and α-neo-endorphin (#58) were used for immunocytochemical staining at 1:500 dilution for 48 hr. Antiserum R-1 was raised against dynorphin A coupled to thyroglobulin via glutaraldehyde, as described for antiserum "Lucia" directed toward dynorphin-(1–13) (9). R-1 exhibits properties indistinguishable from those of "Lucia" under radioimmunoassay conditions. Antiserum 13S was raised in a rabbit by the same coupling method except that dynorphin B was the coupled peptide. In radioimmunoassay studies it crossreacted $<10^{-6}$ with dynorphin A or [Leu]enkephalin. Antiserum #58 against α-neo-endorphin was also produced by coupling the peptide to thyroglobulin via glutaraldehyde. The crossreactivities under immunocytochemical conditions for all three antisera are indicated below. Each antiserum was shown to be specific for its original peptide antigen and was blocked by that peptide at 10 μM but not by the other two at 50 μM or by β_c-endorphin, [Met]enkephalin, [Leu]enkephalin, [Met]enkephalin-Arg^6-Gly^7-Leu^8, [Met]enkephalin-Arg^6-Phe^7, peptide E, or BAM-22P at 50 μM (18–21).

In each of the brain regions studied, adjacent sections were

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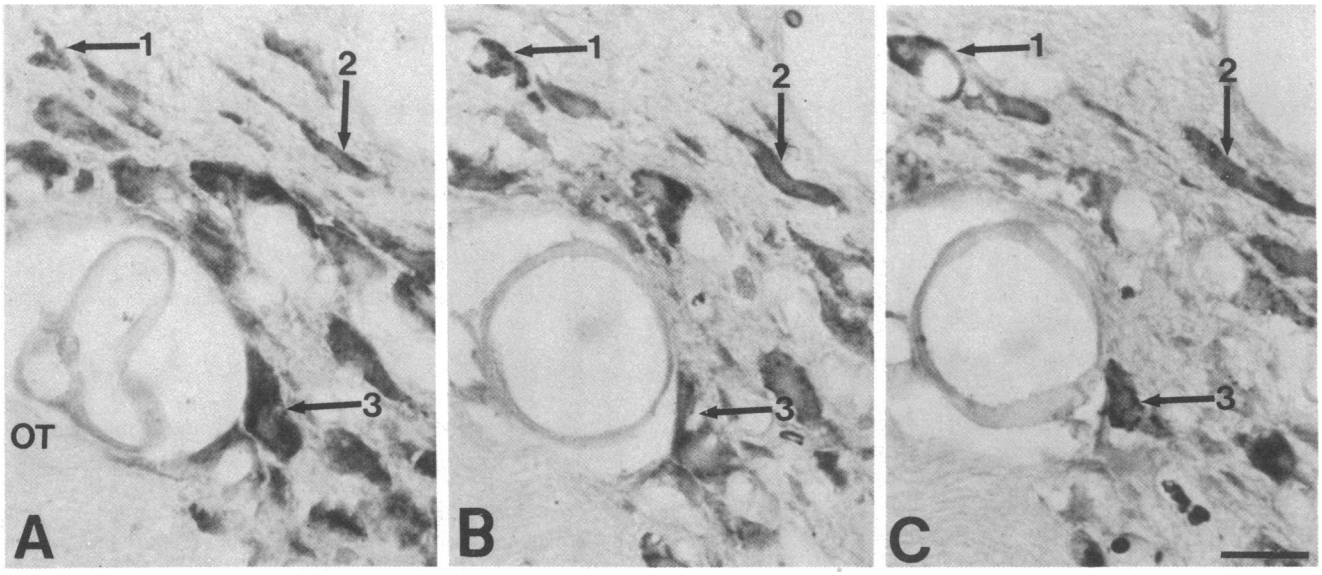

FIG. 1. Supraoptic nucleus of the hypothalamus. (*A*) Cells were stained with dynorphin A antiserum. (*B*) Cells were stained with dynorphin B antiserum. (*C*) Cells were stained with α-neo-endorphin antiserum. In these serial 5-μm sections, several stained cells can be identified in common (the numbered arrows indicate three such cells). Note the common vessel in left middle area of the photographs. Calibration bar, 20 μm.

stained with the three antisera in order to determine whether some cells in the area under study were stained by all three. This type of analysis allowed us to determine that most stained cells in any one area were stained by all three antisera; however, for technical reasons, it was not possible to determine that all cells contained all three immunoreactivities.

RESULTS

Each of the three antisera stained cells in the five specific nuclei—the supraoptic and paraventricular nuclei of the hypothalamus, the lateral hypothalamic area, the nucleus parabrachialis, and the nucleus tractus solitarius—as was suggested by our studies on dynorphin A-positive cells throughout brain (15). After proper alignment of the serial sections within each region, a photographic analysis of the positively stained cells was carried out in order to determine whether any cells were stained with all three antisera.

Within the hypothalamus, the three nuclei were found to have cells stained by all three noncrossreactive antibodies (Figs. 1, 2, and 3): the two nuclei of the magnocellular neurosecretory system, the supraoptic and paraventricular (Figs. 1 and 2); and an unrelated widespread cell group in the lateral hypothalamic area (Fig. 3). The presence of the three types of immunoreactivity (dynorphin A, dynorphin B, and α-neo-endorphin) in magnocellular and nonmagnocellular nuclei suggests at least two discrete neuronal sources in the hypothalamus for pro-dynorphin peptides.

In the more caudal brainstem areas—i.e., nucleus parabrachialis and nucleus tractus solitarius—the three antisera also stained the same cell population (Figs. 4 and 5). The wide sep-

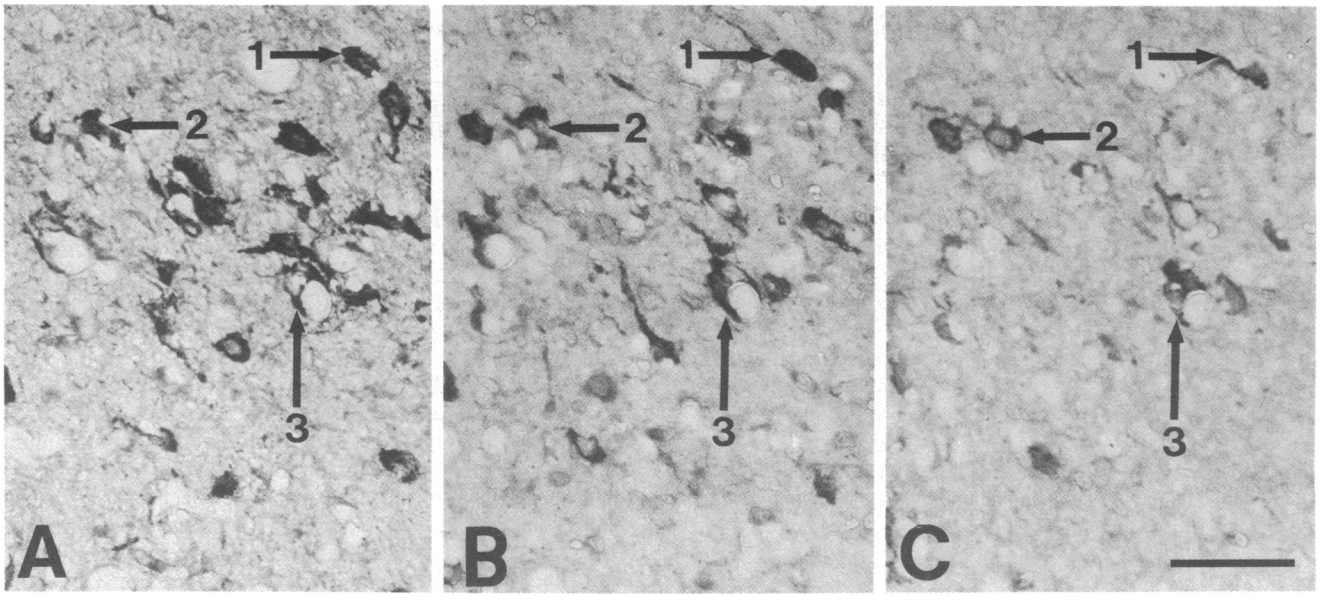

FIG. 2. Paraventricular nucleus of the hypothalamus. *A*, *B*, and *C* as in Fig. 1. In these serial 5-μm sections, stained cells common to all three sections can be seen (numbered cells are common). Calibration bar, 50 μm.

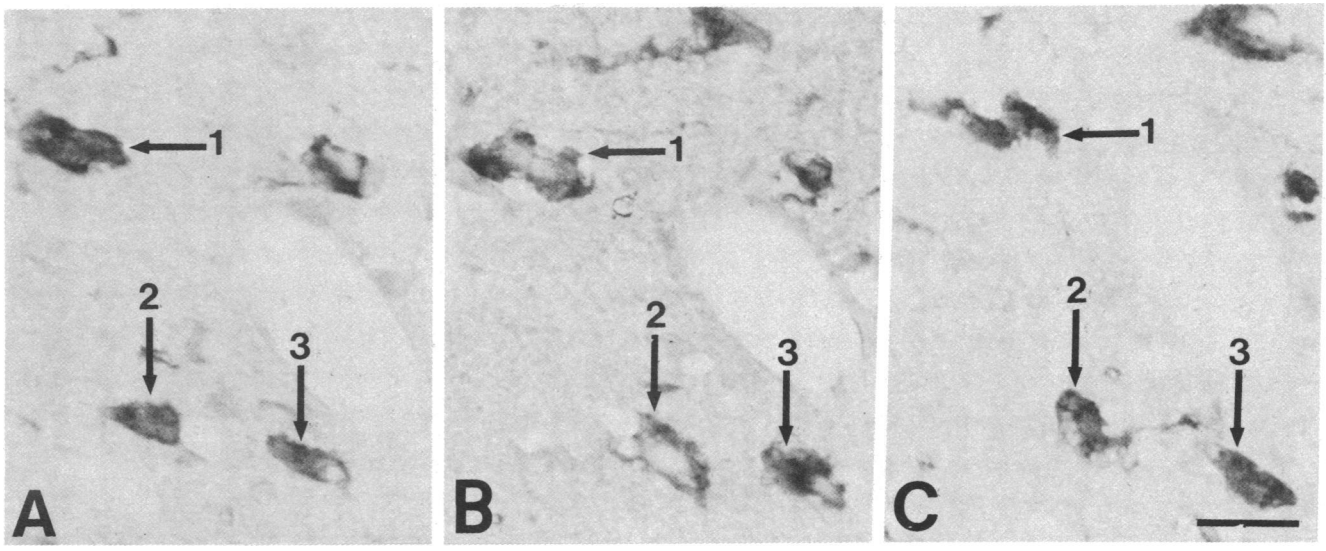

FIG. 3. Lateral hypothalamic area. Three serial 5-μm sections were stained with dynorphin B antiserum (*A*), α-neo-endorphin antiserum (*B*), and dynorphin A antiserum (*C*). The numbered cells are common to all three sections and are stained by the three antisera. Calibration bar, 20 μm.

aration of these two cell groups from each other and from the three hypothalamic cell groups suggests the broad use of pro-dynorphin throughout the neuraxis as a source for these three peptides.

DISCUSSION

The results of this study strongly support the common biosynthetic origin of dynorphin A, dynorphin B, and α-neo-endorphin at several levels in the central nervous system. Still, in light of the great anatomical and biochemical complexity of opioid peptide systems, the three known precursors for these peptides, and the heavy reuse of the Tyr-Gly-Gly-Phe-Leu (or Met) peptide structures, we searched for areas in which one of the three peptides (dynorphin A, dynorphin B, or α-neo-endorphin) might occur without the other two. No example of staining by one antiserum without similar staining by the other two was found, thereby suggesting consistent biosynthesis of all three peptides from pro-dynorphin. However, a broader search throughout nervous tissue is needed to confirm this impression.

Although the antisera used in this work appear to be specific for the peptide sequences under study, it is not possible to determine the form of immunoreactivity seen under immunohistochemical conditions. Thus, it is unclear whether free peptide, biosynthetic intermediate, or precursor is responsible for the staining. It is certainly conceivable that different neuronal groups may process the pro-dynorphin precursor differentially (22) and that our antisera do not permit us to detect this differential processing. However, it is also apparent that either the pro-hormone or the products of the pro-dynorphin precursor are produced in sufficient amounts to allow the visualization of the three types of immunoreactivity in all five cell groups. It thus is most likely that dynorphin A, dynorphin B, and α-neo-endorphin have a common biosynthetic origin both in the endocrine hypothalamic magnocellular–neural lobe system and in the remaining neuronal groups throughout the brain.

It is now clear that there are three families of opioid peptides in the central nervous system—(*i*) the β-endorphin/corticotropin family with a single opioid peptide and three repeated

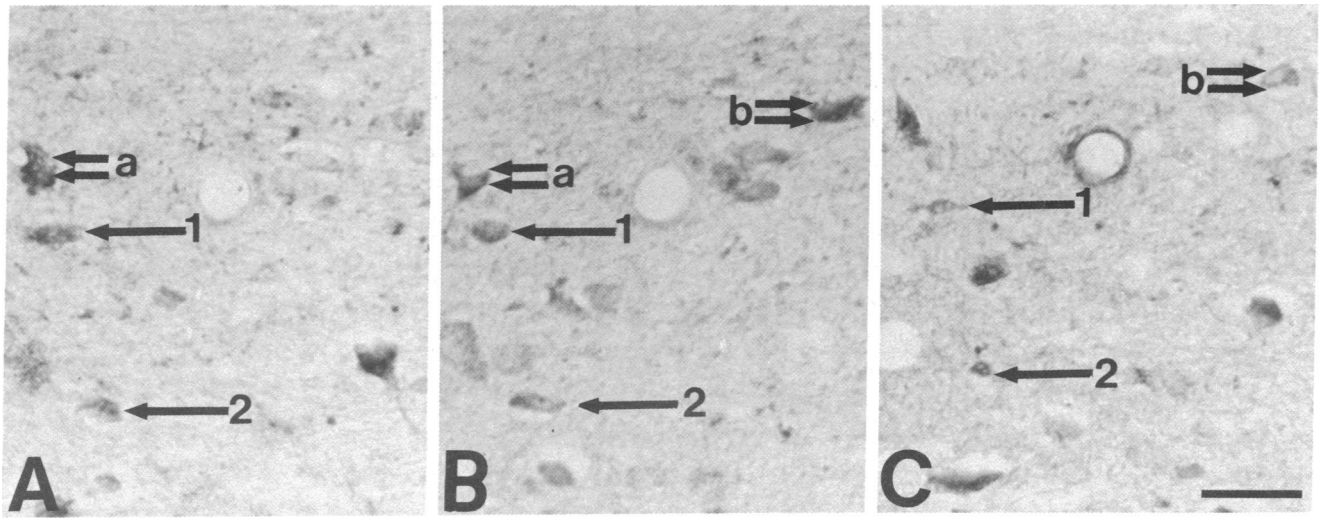

FIG. 4. Nucleus parabrachialis. *A*, *B*, and *C* as in Fig. 1. In these serial 5-μm sections, a few commonly stained cells are numbered or lettered. The lettered double arrows indicate cells seen in only two of the three sections. Calibration bar, 50 μm.

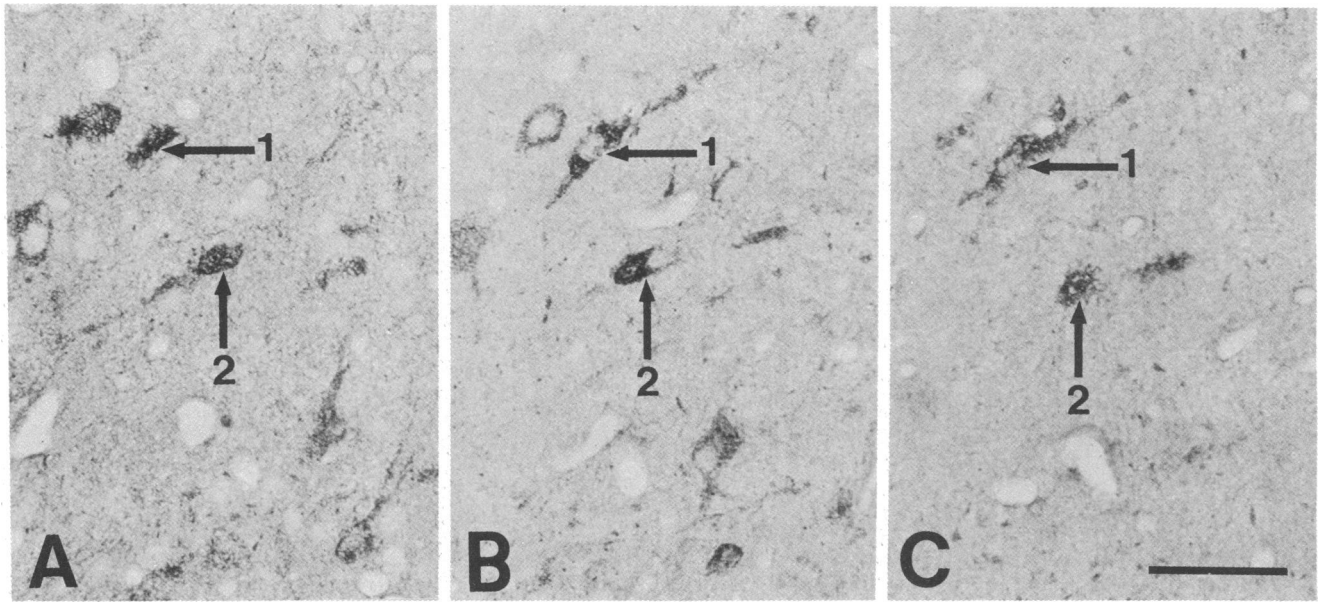

FIG. 5. Nucleus tractus solitarius. *A*, *B*, and *C* as in Fig. 1. In these serial 5-μm sections, two commonly stained cells are numbered. Calibration bar, 50 μm.

melanotropin sequences (23); (*ii*) the enkephalin family with seven opioid peptides, of which six have a [Met]enkephalin core (19–21); and (*iii*) the dynorphin/neo-endorphin family with three opioid peptides that contain the [Leu]enkephalin core (14). The brain β-endorphin system consists primarily of a hypothalamic arcuate cell group with long limbic projections (24, 25), but the two other families exhibit an extensive distribution with multiple cell groups from spinal cord to cortex (26). The current information on the genetic origin of these systems and their general anatomical distribution should lay the groundwork for investigating the local circuitry, the post-translational processing, and the potential functions of these opioid peptide families in the central nervous system.

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